

REMARKS

In a Final Office Action mailed May 11, 2007, the Examiner in charge of the above-identified application objected to and rejected the claims for a variety of reasons. Applicants respond below to the issues presented in the Office Action. In view of the amendments noted above and the arguments presented herein, applicants respectfully request reconsideration of the merits of this application.

Claim Amendments and New Claims

Claim 8 is amended to further clarify that the medium supports growth of undifferentiated human stem cells when osmolarity is in excess of 330 mOsmol as defined by the expression of Oct4, a marker of continued undifferentiated status. Support for this amendment is found for example, at page 6, paragraphs [00029-00038] of the application. No new matter is added.

Also, new Claims 13 and 14 are added to mirror Claims 8 and 9. The new claims further define the undifferentiated human stem cells by expression of Oct4 marker. Oct4 is known to be a marker of continued undifferentiated status. These claims are added to define the scope of the claimed embodiments and to identify as well as establish allowable subject matter. Support for these claims is found, for example, at pg. 5 [00017] and pg. 6 [00029] of the specification. The new claims read on the claims of originally elected Group III. No new matter is added. Based on these amendments, reconsideration of the rejections is kindly requested.

Claim Rejections - 35 USC §103

The rejection to Claims 8, 9 and 12 under 35 U.S.C. § 103(a) in view of Price, et al. is maintained. Specifically, on page 3 of the Action, the Examiner asserts that it would have been a matter of routine optimization to substitute human for mouse embryonic stem cells (ESCs) in a medium having up to about 350mOsm. Further on page 6 of the Action, the Examiner asserts that "Applicants have provided no evidence that the medium of Price would be unsuitable for human stem cells." The Examiner further asserts that a substantive evidentiary showing of unexpected results by applicants is required to overcome this rejection. Applicants traverse this rejection.

In response, applicants submit that in an obviousness inquiry under 35 U.S.C. § 103, the question is whether the claimed invention as a whole would have been obvious, not whether the differences themselves would have been obvious. *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 218 USPQ 871 (Fed. Cir. 1983). Further, an Examiner may not simply pick and choose from one document only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art. See *Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve, Inc.*, 796 F.2d 443 (Fed. Cir. 1986). Here it appears that the Examiner has done just that (i.e., pick and choose) to support an obviousness rejection.

A. Differences between Mouse vs. Human Undifferentiated Stem Cells.

Price et al. as a whole do not render the claims obvious. Price et al. only disclose culture conditions for mouse embryonic stem cells (ESCs) and exemplify only mouse culture techniques. To support applicants' conclusion of non-obviousness, applicants submit that it is generally known in the stem cell field that culture conditions which are optimized for mouse ESCs do not transfer to ESCs from other animal species, much less to human ESCs. The lack of transferability of mouse ESC culturing techniques to human ESCs is widely acknowledged because human ESCs fundamentally and organically behave differently from mouse ESCs. Thus, it would not have been obvious to use conditions worked out for mouse ESCs for culturing human ES cells.

Specifically there are many differences between mouse and human ESCs, which are, in part, due to the phylogenic distance between murine and humans. For example, mouse ESCs, but not human ESCs, have the SSEA-1 marker. Human ESCs exhibit the following markers not present on mouse ESCs: SSEA-3, SEA-4, TRA-1-60, and TRA-1-81. Also, conditions that sustain mouse ESC cultures and support mouse ESC differentiation do not equally support human ESC cultures. ESCs from mice and humans require distinct sets of factors to remain undifferentiated. Also, factors (including LIF and WNT) that are sufficient for sustaining pluripotency in the murine ESC system are not effective for maintaining undifferentiation of human ESCs.

Notably, mouse ESCs require LIF in the culture media to remain undifferentiated. Human ESCs have no LIF receptor, so providing LIF in primate ESC culture media does not maintain cells in an undifferentiated state. Further, the leukemia inhibitory factor (LIF)/Stat3 pathway, a key to mouse ESC proliferation, does not support human ESC proliferation and appears inactive in conditions that support human ESCs (Daheron L, et al., LIF/STAT3 signaling fails to maintain self-renewal of human embryonic stem cells, *Stem Cells* (2004) 22:770-778).

Further, bone morphogenetic proteins (BMPs) together with LIF support mouse ESC self-renewal at clonal densities in serum-free medium (Ying Q L, et al., *Cell* (2003) 115:281-292). In human ESCs, however, BMPs cause rapid differentiation to trophoblast cells in conditions that would otherwise support self-renewal (Xu RH, et al., *Nat Biotechnol* (2002) 20:1261-1264). Also, FGF signaling is important to self-renewal of human ESCs, but not from mice (Xu RH, et al. Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ESCs. *Nat Methods* (2005) 2:185-190). These basic differences between mouse and human ESCs help to show that at the time of filing, a skilled person would have had no reasonable expectation that the methods for mouse ESC expansion would equally support culturing and sub-culturing (i.e., cloning) human ESCs.

B. Industry Recognizes Differences Between Mouse vs. Human undifferentiated Stem Cells.

Indeed, murine stem cells have a nutritional requirement that differs from human ESCs (i.e., prefer to grow at a lower osmolarity than rhesus or human ESCs). As evidence, applicants refer to marketing materials from Invitrogen relating to their "Stem Cell research products and Services," in particular KnockOut™ DMEM and Serum Replacement (Invitrogen Article disclosed in Supplemental Information Disclosure Statement, herewith). This article indicates that " KnockOut™ DMEM has reduced osmolarity to mimic the natural embryonic tissue, thus improving the morphology of the cells and reducing cell differentiation (Figure 3)." Upon closer examination, it appears that the cells in Figure 3 are murine ESCs. Figure 3 is a bar graph comparing only murine D3 ES cells cultured at low density in KnockOut™ DMEM.

Moreover, Thermo Fischer Scientific recently launched a new line of cell culture products (HyClone Advance STEM™), which support expansion and maintenance of undifferentiated

murine embryonic and human adult mesenchymal stem cells for directed differentiation of such differentiated cells into adipocytes, chondrocytes and osteocytes. (See, June 28, 2007 Press release of the HyClone Advance STEM™ product line, attached herewith in a Supplemental Information Disclosure Statement). Notably, Thermo Fischer Scientific does not recommend using this product (through its silence) for culturing human ESCs. This tells the researcher that mouse ESCs and adult human differentiated cells can use the same stem cell medium, but human ESCs can't! The press release goes on to say that a specific HyClone Advance Stem™ Low Osmo DMEM has been developed featuring an optimized osmolarity approximating that of murine embryonic tissue. Again, this shows that the culture conditions for murine ESCs, which mimic adult human differentiated cells, are different from human ESCs. Therefore, medium optimized for mouse ESCs is not optimal for human cells.

Applicants submit that developing a culture system optimal for human ESCs is not a trivial task and requires much trial and error, particularly when using the mouse ESC culture conditions as a starting point. As such, it would not have been obvious, nor simply a matter of routine optimization, to a skilled artisan to substitute mouse ESC culture conditions for human.

C. Price et al. Disclose Culturing Mouse ESCs And Are Silent On Medium Quality.

Price et al. simply disclose osmolarity as one of many variables that can be modified in a medium for maintaining cells in an undifferentiated state. Price et al. do not mention any particular species in connection with osmolarity. However, after careful review, it is understood that Price et al. disclose methods for maintaining undifferentiated mouse ESCs, not human cells.

Specifically, Price et al. provide that

"[P]referably, the osmolarity of the 1X medium is between about 280 and 310 mOsmol. However, osmolarity of the 1X medium can be as low as about 260 mOsmol and as high as about 350 mOsmol." (see, para. 101).

Price et al. disclose a broad range of osmolarity without "*sufficient specificity*" for the osmolarity when culturing human ESCs. Applicants maintain that deducing an optimal osmolarity concentration for human ESCs is not a routine task, as it is not the same as mouse ESCs or adult human cells noted above. Osmolarity is not recited anywhere in the working examples or even in the claims. Clearly, Price et al. were not convinced that osmolarity was an important

consideration in designing and preparing media for even mouse ESCs (much less human ESCs) or they would have exemplified it or claimed it. The manner and context in which osmolarity is disclosed as well as the known species-specific differences in the culture conditions (some of which are noted hereinabove) would not have motivated a skilled person to apply mouse ESC culture conditions to a human ESC culture with an expectation of success. In fact, there would not have been any success as human ESCs have a higher osmolarity requirement than mouse ESCs, as discussed herein.

Further, Price et al. go on to provide that for ESCs, the LIF concentration is about 10 ng/mL (see, para. 101). As indicated herein above, it is well known that LIF is used in media for maintaining mouse ESCs in an undifferentiated state, not human ESCs. In fact, all of the examples in Price et al. are directed to culturing mouse ESCs using LIF. In the paragraph following the brief discussion about relative osmolarity, Price et al. provides

"[T]he serum-free supplement and the medium of the present invention can be used to culture ES cells derived from a number of animals, including human, monkey, ape, mouse, rat, hamster, rabbit, guinea pig, cow, swine, dog, horse, cat, goat, sheep, bird, reptile, amphibian, and fish." (See, Price et al., para. 102).

Based on the differences set forth above between mouse and human ESCs, one skilled in the art would not reasonably expect to apply the protocols and culture conditions slated for mouse ESCs on human ESCs with success.

Another important difference between Price et al. and applicants' disclosure relates to measurement of the medium quality. Nowhere in Price et al. is there mention of how cell numbers were statistically measured or medium quality as measured by medium quality index (MQI). It appears that Price et al. only looked at relative cell numbers (see Price, pg. 8, 13 and 14) and not MQI. However, relative cell number is not the best indicator of a medium having a suitable level of osmolarity for maintaining human stem cells in an undifferentiated state. If one were to have followed Price's method of determining cell viability, one would use only relative cell number. If this is done, one would be led to believe that 310 mOsMol (see present application Fig. 3) is the best osmolarity. Based on Price's use of cell number, one would have been *led away* from using an excess of 330 mOsMol and preferably about 350 mOsMol for maintaining human stem cells in an undifferentiated state, as defined by continued Oct4 expression. This is a surprising result given that *normal human serum* has a physiological

Application No.: 10/811,423
Response dated: September 11, 2007
Reply to Office Action dated: May 11, 2007

osmolarity of 290 mOsMol (see page 3, [00012] and page 6 [00038] of the present specification). Thus, Price et al. do not render the claims obvious. Applicants believe that to make an obviousness rejection, the Examiner has selectively chosen from Price et al. only enough to support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art.

Applicants have made a diligent effort to place the claims in condition for allowance. However, should there remain unresolved issues that require adverse action, it is respectfully requested that the Examiner telephone applicants' attorney at the number listed below so that such issues may be resolved as expeditiously as possible.

For the reasons stated above, and in view of the above amendments, this application is now considered to be in condition for allowance and such action is earnestly solicited.

Fees

A Petition for an extension of time and a Request for Continued Examination (RCE) accompany this response. Please charge the fees to Deposit Account No. 17-0055. No other fees are believed due in regard to this submission. If any other fee is due in this or any subsequent response, please consider this to be a petition for the appropriate extension and a request to charge the petition fee to the Deposit Account No. 17 0055.

Respectfully submitted,



Sara D. Vinarov
Reg. No. 48,524
Attorney for Applicants
QUARLES & BRADY LLP
P.O. Box 2113
Madison, WI 53701-2113

TEL (608) 251-5000
FAX (608) 251-9166